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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

As the below named inventor(s), I/we declare that:

This declaration is directed to:

- ☒ The attached application, or
☐ Application No. _____, filed on _____,
☐ as amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including material information which became available between the filing date of the prior application and the National or PCT International filing date of the continuation-in-part application, if applicable; and

All statements made herein of my/own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)Inventor one: James R. Connor Date: 2-9-01Signature:  Citizen of: United States of AmericaInventor two: Zheng Ye Date: _____Signature: _____ Citizen of: China

Inventor three: _____ Date: _____

Signature: _____ Citizen of: _____

Inventor four: _____ Date: _____

Signature: _____ Citizen of: _____

☒ Additional inventors are being named on 2 additional form(s) attached hereto.

Burden Hour Statement: This collection of information is required by 35 U.S.C. 116 and 37 CFR 1.63. The information is used by the public to file (and the PTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This form is estimated to take 1 minute to complete. This time will vary depending upon the needs of the individual cases. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

empirically. The conditions of the reaction must also be compatible with full activity of the polymerase.

It is the usual practice to set up the reaction at room temperature and to begin it with a 92-96°C denaturation step. It has been suggested that even while the samples are being prepared primer extension by the Taq DNA polymerase could occur. At room temperature there would be little specificity to primer-template interactions. Experiments have shown that some of the nonspecific amplification products can be eliminated under so-called "hot start" conditions. This approach keeps the sample at a temperature greater than the calculated annealing temperature for the specific primer before the reaction is started.

Details of the Reaction

In addition to a genomic DNA sample usually containing less than 1 (pmol) of specific target sequence, the 25-100 μ liter volume includes 20 nmol of each of the four deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), 10 to 100 pmol of each primer, the appropriate salts and buffers and DNA polymerase. The nucleotide concentration must be sufficient to saturate the enzyme, but not so low or unbalanced as to promote misincorporation (see below). The primer concentration must be high enough to anneal rapidly to the single-stranded target and, in later stages of the reaction, faster than target-target reassociation. Temperature control and timing are also important. Denaturation must be efficient, but the temperature must not be too high or held for too long a period, because the Taq polymerase, although heat-resistant, is not indefinitely stable. The temperature used for annealing must maximize specific primer annealing and polymerase elongation but not sacrifice yield by reducing primer-template hybridization.

The reaction mixture is usually overlaid with mineral oil to prevent evaporation, thereby contributing to rapid thermal equilibration and eliminating a concentration of reagents during the course of the reaction. A newly designed thermocycler is capable of very rapid temperature change, and